

## Claims

1. A method of cloning a non-human mammal, said method comprising the steps of:

(a) incubating a permeabilized cell in a reprogramming media under conditions that allow the removal of a factor from a nucleus, chromatin mass, or chromosome of said permeabilized cell or the addition of a factor from said reprogramming media to said nucleus, chromatin mass, or chromosome, thereby forming a reprogrammed cell;

(b) inserting said reprogrammed cell into a nucleated or enucleated oocyte, thereby forming a nuclear transfer oocyte; and

(c) transferring said nuclear transfer oocyte or an embryo formed from said nuclear transfer oocyte into the uterus of a host mammal under conditions that allow said nuclear transfer oocyte or said embryo to develop into a fetus.

2. The method of claim 1, wherein said reprogramming media is a cell extract.

3. The method of claim 1, wherein the nucleus of said permeabilized cell remains membrane-bounded and the chromatin in said nucleus does not condense during incubation in said reprogramming media.

4. The method of claim 1, wherein a chromatin mass is formed from incubation of said permeabilized cell in said reprogramming media.

5. The method of claim 1, wherein said reprogrammed cell is incubated under conditions that allow the membrane of said reprogrammed cell to reseal.

6. The method of claim 1, wherein said reprogrammed cell is purified from said reprogramming media prior to insertion into said nuclear transfer oocyte.

7. The method of claim 1, wherein said fetus develops into a viable offspring.

8. The method of claim 1, wherein said nuclear transfer oocyte from step (b) is cultured under conditions that allow cell division and one of the resulting cells is recloned one or more times.

5           9. The method of claim 1, wherein said permeabilized cell and said nuclear transfer oocyte are from the same species.

10           10. The method of claim 1, wherein said non-human mammal is a cow, sheep, rabbit, pig, mouse, rat, goat, or buffalo.

11. The method of claim 10, wherein said non-human mammal is a cow.

12. The method of claim 1, wherein said permeabilized cell is a fibroblast, epithelial cell, neural cell, epidermal cell, keratinocyte, hematopoietic cell, melanocyte, chondrocyte, B-lymphocyte, T-lymphocyte, erythrocyte, macrophage, monocyte, muscle cell, embryonic stem cell, embryonic germ cell, fetal cell, placental cell, or embryonic cell.

13. The method of claim 1, wherein said permeabilized cell is a cell of the female reproductive system.

14. The method of claim 13, wherein said permeabilized cell is a mammary gland, ovarian cumulus, granulosa, or oviductal cell.

15. The method of claim 1, wherein said nuclear transfer oocyte from step (b) expresses lamin A, lamin C, or NuMA protein at a level that is less than 5 fold greater than the corresponding level expressed by a control oocyte from the same species.

16. A method of cloning a non-human mammal, said method comprising the steps of:

(a) contacting a donor nucleus that has less than four sets of homologous

chromosomes with a reprogramming media under conditions that allow formation of a chromatin mass without causing DNA replication;

(b) inserting said chromatin mass into an oocyte, thereby forming a nuclear transfer oocyte; and

5 (c) transferring said nuclear transfer oocyte or an embryo formed from said nuclear transfer oocyte into the uterus of a host mammal under conditions that allow said nuclear transfer oocyte or said embryo to develop into a fetus.

10 17. The method of claim 16, wherein reprogramming media is a mitotic extract, detergent and salt solution, a detergent solution, a salt solution, or protein kinase solution.

18. The method of claim 16, wherein said chromatin mass from step (a) is purified from said extract prior to insertion into said nuclear transfer oocyte.

15 19. The method of claim 16, wherein said fetus develops into a viable offspring.

20 20. The method of claim 16, wherein said nuclear transfer oocyte from step (b) is cultured under conditions that allow cell division and one of the resulting cells is recloned one or more times.

21. The method of claim 16, wherein said donor nucleus and said nuclear transfer oocyte are from the same species.

25 22. The method of claim 16, wherein said non-human mammal is a cow, sheep, rabbit, pig, mouse, rat, goat, or buffalo.

23. The method of claim 22, wherein said non-human mammal is a cow.

30 24. The method of claim 16, wherein said donor nucleus is diploid.

25. The method of claim 16, wherein said donor nucleus is from a fibroblast, epithelial cell, neural cell, epidermal cell, keratinocyte, hematopoietic cell, melanocyte, chondrocyte, B-lymphocyte, T-lymphocyte, erythrocyte, macrophage, monocyte, fibroblast, muscle cell, embryonic stem cell, embryonic germ cell, fetal cell, placental cell, or embryonic cell.

26. The method of claim 16, wherein said donor nucleus is from a cell of the female reproductive system.

27. The method of claim 26, wherein said cell of the female reproductive system is a mammary gland, ovarian cumulus, granulosa, or oviductal cell.

28. The method of claim 16, wherein said oocyte from step (b) expresses lamin A, lamin C, or NuMA protein at a level that is less than 5 fold greater than the corresponding level expressed by a control oocyte from the same species.

29. A method of cloning a non-human mammal, said method comprising the steps of:

(a) inserting a cell, nucleus, or chromatin mass into an oocyte, thereby forming a first embryo;

(b) contacting one or more cells from said first embryo with one or more cells from a second embryo, thereby forming a third embryo; wherein said second embryo is an *in vitro* fertilized embryo, naturally-occurring embryo, or parthenogenetically activated embryo, and wherein at least one of said first embryo and said second embryo is a compaction embryo; and

(c) transferring said third embryo into the uterus of a host mammal under conditions that allow said third embryo to develop into a fetus.

30. A method of cloning a non-human mammal, said method comprising the steps of:

(a) inserting a cell, nucleus, or chromatin mass into an oocyte, thereby forming a

first embryo;

(b) contacting one or more cells from said first embryo with one or more cells from a second embryo, thereby forming a third embryo; wherein said second embryo is an *in vitro* fertilized embryo, naturally-occurring embryo, or parthenogenetically activated embryo, and wherein said first embryo and said second embryo are at different cell-stages; and

(c) transferring said third embryo into the uterus of a host mammal under conditions that allow said third embryo to develop into a fetus.

31. A method of cloning a non-human mammal, said method comprising the steps of:

(a) contacting a donor nucleus with a reprogramming media under conditions that allow formation of a chromatin mass;

(b) inserting said chromatin mass into an oocyte, thereby forming a first embryo;

(c) contacting one or more cells from said first embryo with one or more cells from a second embryo, thereby forming a third embryo; wherein said second embryo is an *in vitro* fertilized embryo, naturally-occurring embryo, or parthenogenetically activated embryo; and

(d) transferring said third embryo into the uterus of a host mammal under conditions that allow said third embryo to develop into a fetus.

32. The method of claim 31, wherein step (a) involves contacting a donor nucleus that has less than four sets of homologous chromosomes with a reprogramming media under conditions that allow formation of a chromatin mass without causing DNA replication.

33. A method of cloning a non-human mammal, said method comprising the steps of:

(a) incubating a permeabilized cell in a reprogramming media under conditions that allow the removal of a factor from a nucleus, chromatin mass, or chromosome of said permeabilized cell or the addition of a factor from said reprogramming media to said

nucleus, chromatin mass, or chromosome, thereby forming a reprogrammed cell;

(b) inserting said reprogrammed cell into an oocyte, thereby forming a first embryo;

(c) contacting one or more cells from said first embryo with one or more cells from a second embryo, thereby forming a third embryo, wherein said second embryo is an *in vitro* fertilized embryo, naturally-occurring embryo, or parthenogenetically activated embryo; and

(d) transferring said third embryo into the uterus of a host mammal under conditions that allow said third embryo to develop into a fetus.

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34. The method of claim 33, wherein said reprogrammed cell is incubated under conditions that allow the membrane of said reprogrammed cell to reseal.

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35. The method of claim 31 or 33, wherein said reprogramming media is a cell extract.

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36. The method of any one of claims 29-31 and 33, wherein said fetus develops into a viable offspring.

37. The method of any one of claims 29-31 and 33, wherein at least 10% cells in the placenta of said fetus are derived from said second embryo.

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38. The method of any one of claims 29-31 and 33, wherein at least 50% cells in the fetal tissue of said fetus are derived from said first embryo.

39. The method of any one of claims 29-31 and 33, wherein said non-human mammal is a cow, sheep, rabbit, pig, mouse, rat, goat, or buffalo.

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40. The method of claim 39, wherein said non-human mammal is a cow.

41. The method any one of claims 29-31 and 33, wherein said third embryo

expresses lamin A, lamin C, or NuMA protein at a level that is less than 5 fold greater than the corresponding level expressed by a control oocyte from the same species.

- 5 42. The method any one of claims 29-31 and 33, wherein said cells from said second embryo are injected into said first embryo between the zona pellucida and the embryonic cells.

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